

**AMENDED CLAIMS**

[received by the International Bureau on 29 June 2001 (29.06.01);  
original claim 2 replaced by new claim 2;  
original claims 3-40 renumbered as claims 3-41 (4 pages)]

1. An integration and expression plastid vector competent for stably transforming the plastid genome to confer stress tolerance which comprises an expression cassette which  
5 comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a spacer sequence, a promoter operative in said plastid, a selectable marker sequence, a DNA sequence encoding an osmoprotectant, at least one restriction site for the insertion of a heterologous target DNA sequence, a transcription termination region functional in said plastid, and the 3' part of the plastid DNA sequence inclusive of a spacer sequence.
- 10 2. An integration and expression plastid vector competent for stably transforming the plastid genome to confer stress tolerance which comprises an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species, a promoter operative in said plastid, a selectable marker sequence, a DNA sequence  
15 encoding an osmoprotectant, at least one restriction site for the insertion of a heterologous target DNA sequence, a transcription termination region functional in said plastid, and the 3' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species.
3. The vector of claim 2 further comprising a heterologous DNA sequence which  
20 codes for a molecule of interest that is inserted in one of the restriction sites.
4. The vector of claim 3 where the molecule of interest is a polypeptide.
5. A vector of claim 3, wherein said vector further comprises a ribosome binding site and a 5' untranslated region (5' UTR) to enhance expression.
6. A vector of claim 3 wherein the osmoprotectant is selected from a group  
25 consisting of sugars, sugar alcohols, sugar derivatives, and amino acids including proline and glycine-betaine.
7. A vector of claim 6 wherein the osmoprotectant is trehalose.
8. A vector of claim 6 wherein the trehalose is at least one of the complex TPS1, TPS2, TPS3 or TSL1.
- 30 9. The vector of claim 3 wherein the osmoprotectant is selected from a group consisting of TSP1, *E. Coli* otsA, stachyose, and ononitol.
10. The vector of claim 6 wherein the osmoprotectant is a sugar.
11. The vector of claim 10, wherein the sugar is a monosaccharide including but not limited to fructose.

12. The vector of claim 10, wherein the sugar is a disaccharide including but not limited to sucrose.
13. The vector of claim 10, wherein the sugar is a trisaccharide including but not limited to raffinose.
- 5 14. The vector of claim 10 wherein the sugar is dulcitol.
15. The vector of claim 6 wherein the osmoprotectant is a sugar alcohol.
16. The vector of claim 15 wherein the sugar alcohol is a polyhyric alcohol.
17. The vector of claim 16 wherein the polyhyric alcohol is a trihydric alcohol including but not limited to glucoglycerol.
- 10 18. The vector of claim 16 wherein the polyhyric alcohol is a tetrahydric alcohol including but not limited to erythritol.
19. The vector of claim 16 wherein the polyhyric alcohol is a hexahydric alcohol including but not limited to mannitol or sorbitol.
- 20 A vector of claim 3 wherein at least one DNA encodes a component of trehalose synthase that is under the control of a promoter to produce a transgenic plant.
- 15 21. The vector of claim 20 wherein the promoter is constitutive.
22. The vector of claim 20 wherein the promoter is tissue specific, light-induced, or stress-induced.
23. A stably transformed plant which has been transformed by the vector of any one of claims 3, wherein the transformed plant is more tolerant of stresses selected from a group consisting of water-deprivation, freezing, salt, heat and cold than is the untransformed plant.
- 20 24. The plant of claim 23 wherein the plant does not include target DNA.
25. A stably transformed plant of claim 23, or the progeny thereof including seeds, wherein said plant display no negative pleiotropic effects.
- 25 26. A transgenic plant of claim 23, wherein the plant is a transgenic plant which is morphologically indistinguishable from an untransformed plant.
27. A transgenic plant of claim 23, wherein the plant is a solanaceous plant edible for a mammal.
28. A transgenic plant of claim 23, wherein the plant is a crop plant edible for a mammal.
- 30 29. A transgenic plant of either claim 27 or 28, wherein the mammal is a human.
30. A transgenic plant of claim 23, wherein the plant is a monocotyledonous plant selected from the group of rice, wheat, grass, rye, barley, oat, and maize.

31. A transgenic plant of claims 23, wherein the plant is a dicotyledonous plant selected from the group of soybean, peanut, grape, sweet potato, pea, canola, tobacco, tomato and cotton.

32. A transgenic plant of claim 23, wherein the plant is tobacco, tomato, potato, rice, brassica, cotton, maize and soybean.

33. A method of conferring drought resistance to plants, said method comprising introducing into the plastid of plant species that are susceptible to water stress, an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species, a promoter operative in said plastid, a DNA sequence encoding a gene which confers osmoprotection, a heterologous DNA sequence encoding a molecule of interest, a selectable marker sequence, a transcription termination region functional in said plastid, and a 3' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species.

34. The method of claim 33, wherein said method further comprises culturing said plant in a plant growth medium containing an effective amount of polyethylene glycol (PEG) for selection, and selecting transformed plant cells capable of growth in said plant growth medium.

35. The method of claim 34, wherein said method further comprises regenerating the selected transformed plant cells into stable transgenic plants.

36. A method of increasing trehalose accumulation in plant cells thereby conferring osmotic stress resistance to said plant cells, where said method comprises introducing to the plastid of plant species that are susceptible to osmotic stress an expression cassette which comprises as operably joined components, a 5' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species, a promoter operative in said plastid, a DNA sequence encoding the Yeast T6P synthase (TSP) gene which confers drought resistance, a heterologous DNA sequence encoding a molecule of interest, a selectable marker sequence, a transcription termination region functional in said plastid, and a 3' part of the plastid DNA sequence inclusive of a transcriptionally active spacer sequence that is conserved in the plastid genome of different plant species.

37. The method of claim 36, wherein said method further comprises culturing said plant in a plant growth medium containing an effective amount of polyethylene glycol (PEG) for selection, and selecting transformed plant cells capable of growth in said plant growth medium.

38. The method of claim 37, wherein said method further comprises regenerating the selected transformed plant cells into stable transgenic plants.

39. The vector of claim 2, wherein said plastid is a chloroplast.

40. The vector of claim 39, wherein the vector is a universal chloroplast vector.
41. The methods of claims 32 or 35, wherein the plastid is a chloroplast.
42. The vector of claim 2, wherein the transcriptionally active spacer sequence comprises a portion of the intergenic spacer 2 region between and inclusive of the tRNA<sup>Ile</sup> and the tRNA<sup>Ala</sup> genes of a chloroplast genome.
- 5 43. The vector of claim 42, wherein the spacer region is located in an inverted repeat of the chloroplast genome.